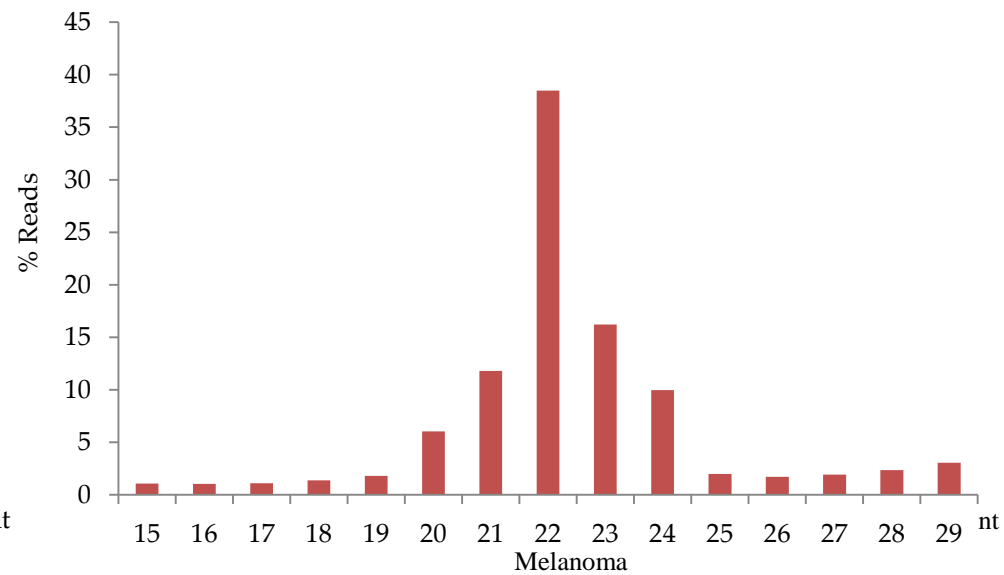
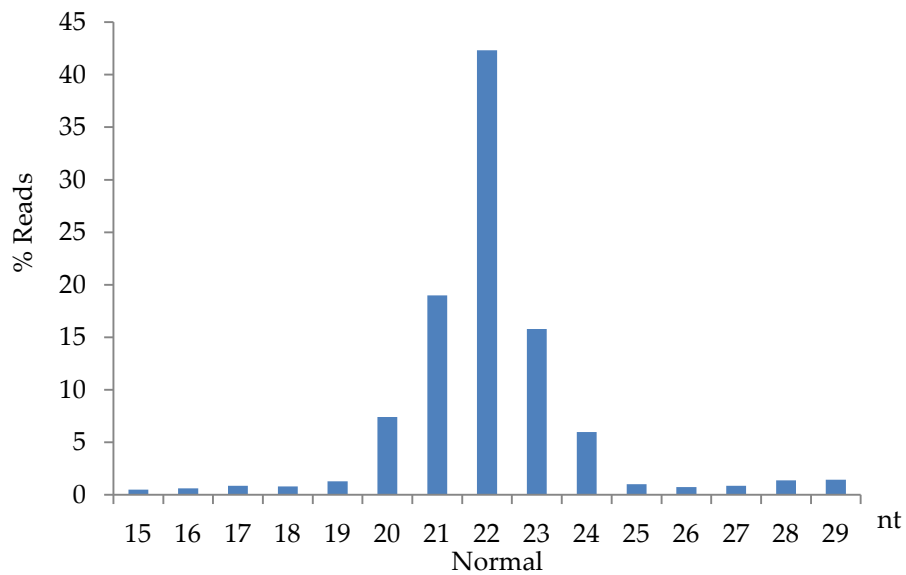


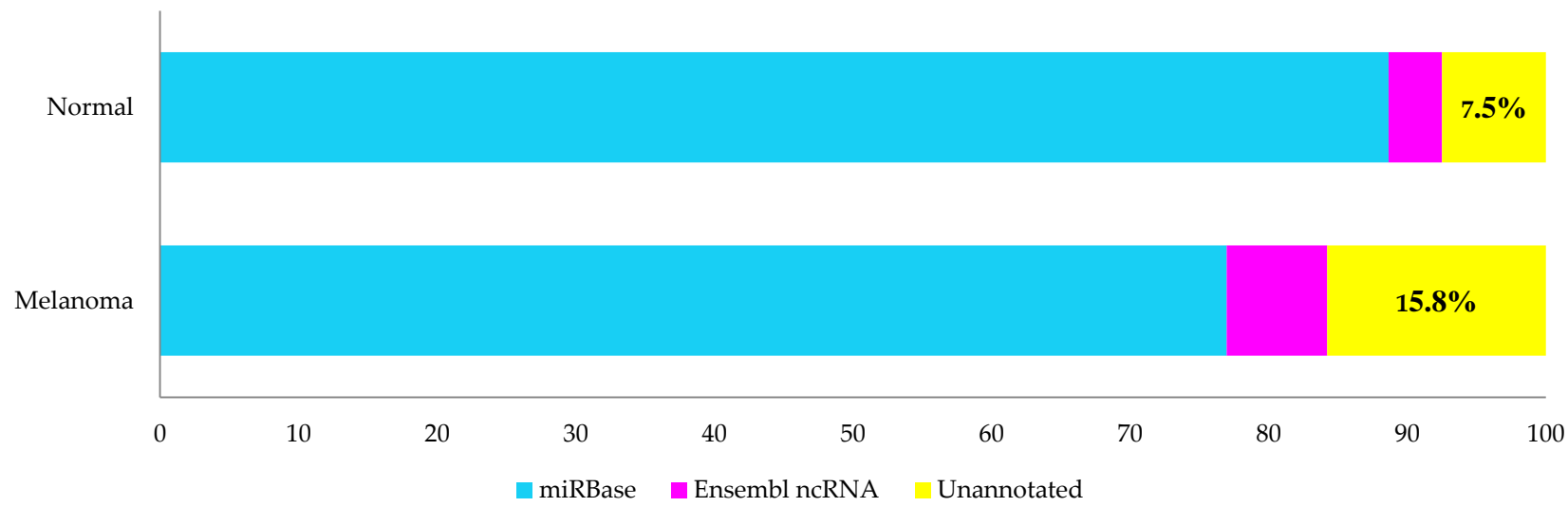
## MiRNA profile in canine oral melanoma

Md. Mahfuzur Rahman, Yu-Chang Lai, Norio Ushio, Al Asmaul Husna, Hui-wen Chen, Yukiko Tanaka, Noriaki Miyoshi, Takayuki Nakagawa, Ryuji Fukushima, Naoki Miura

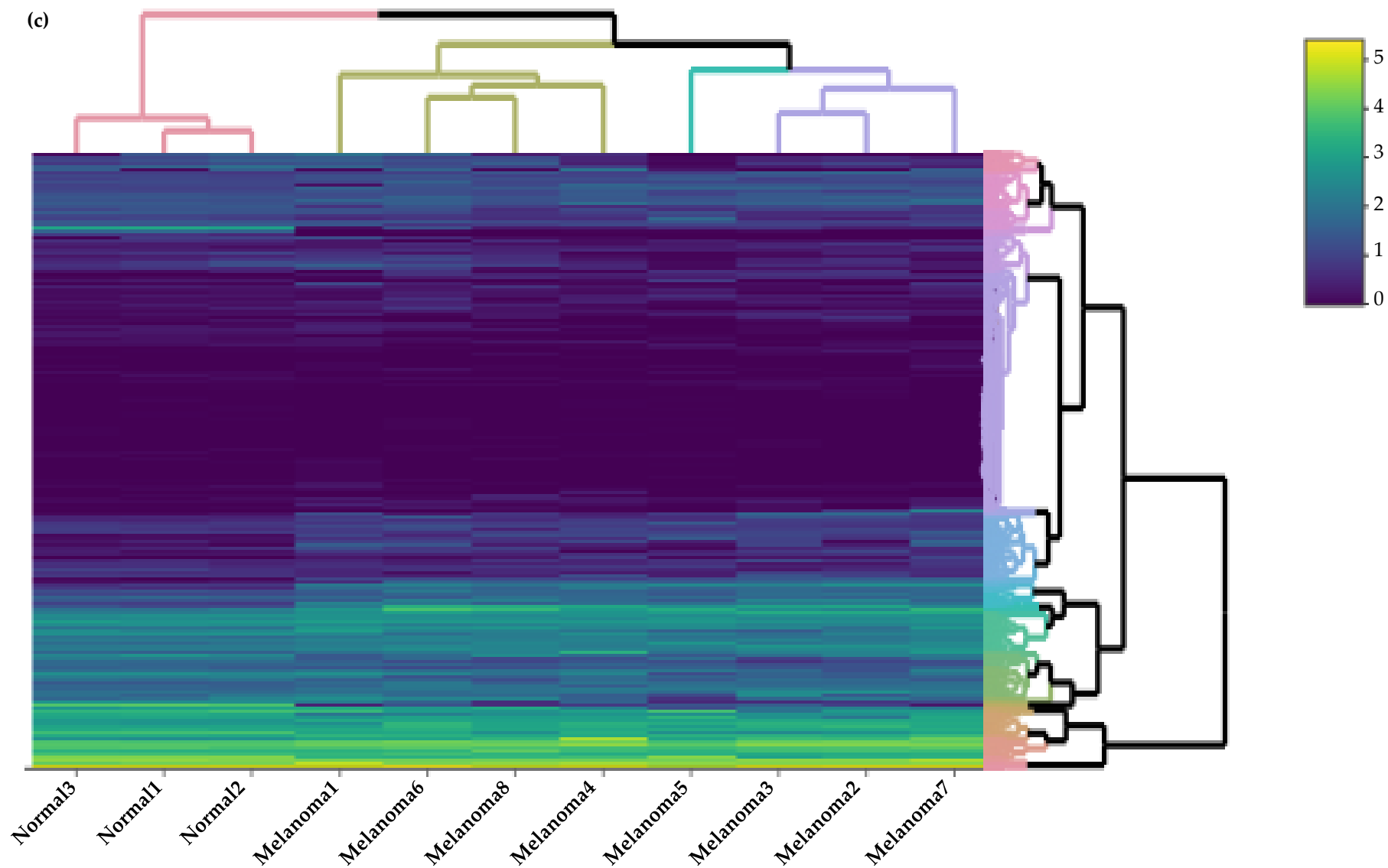
(a)



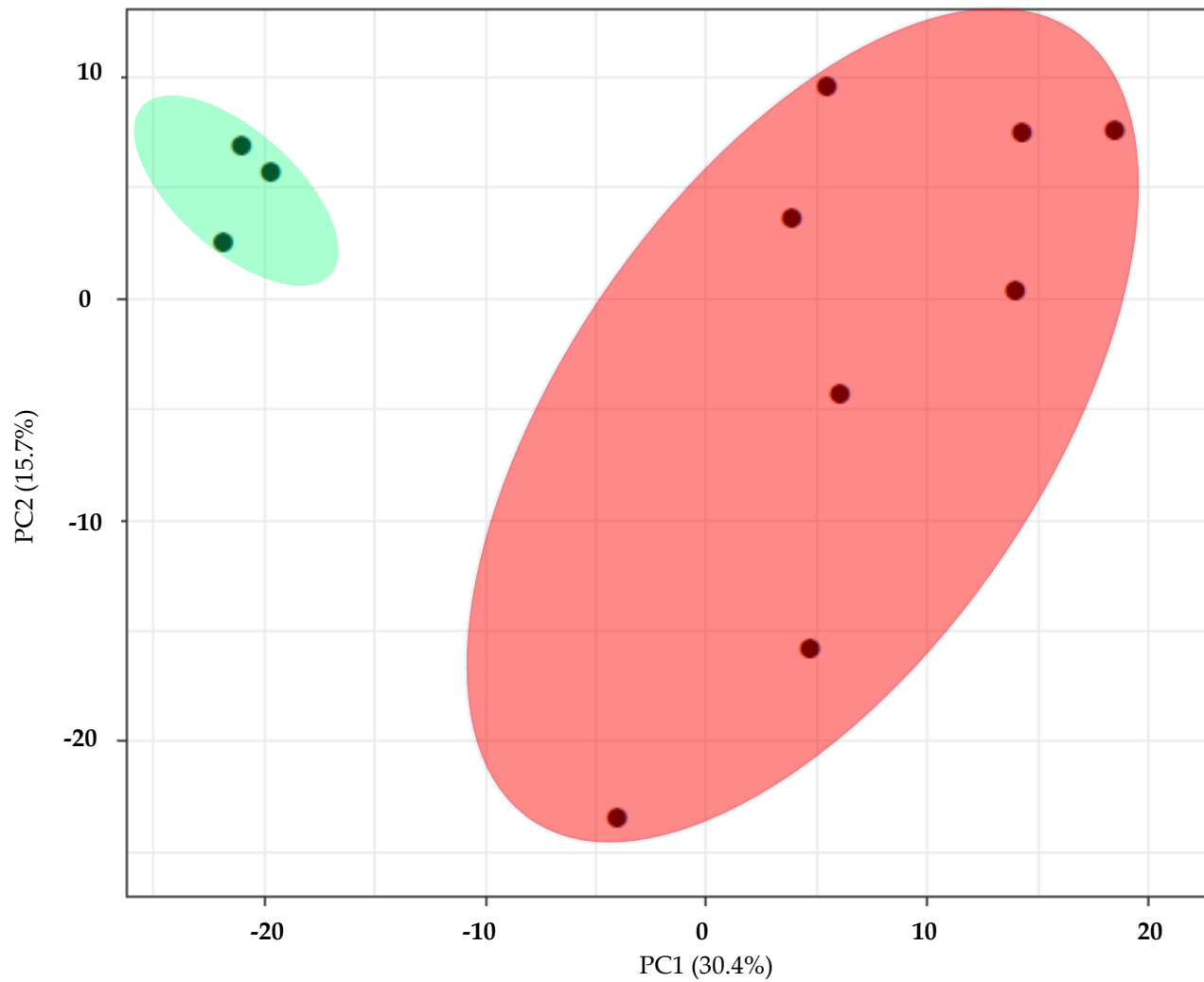
(b)



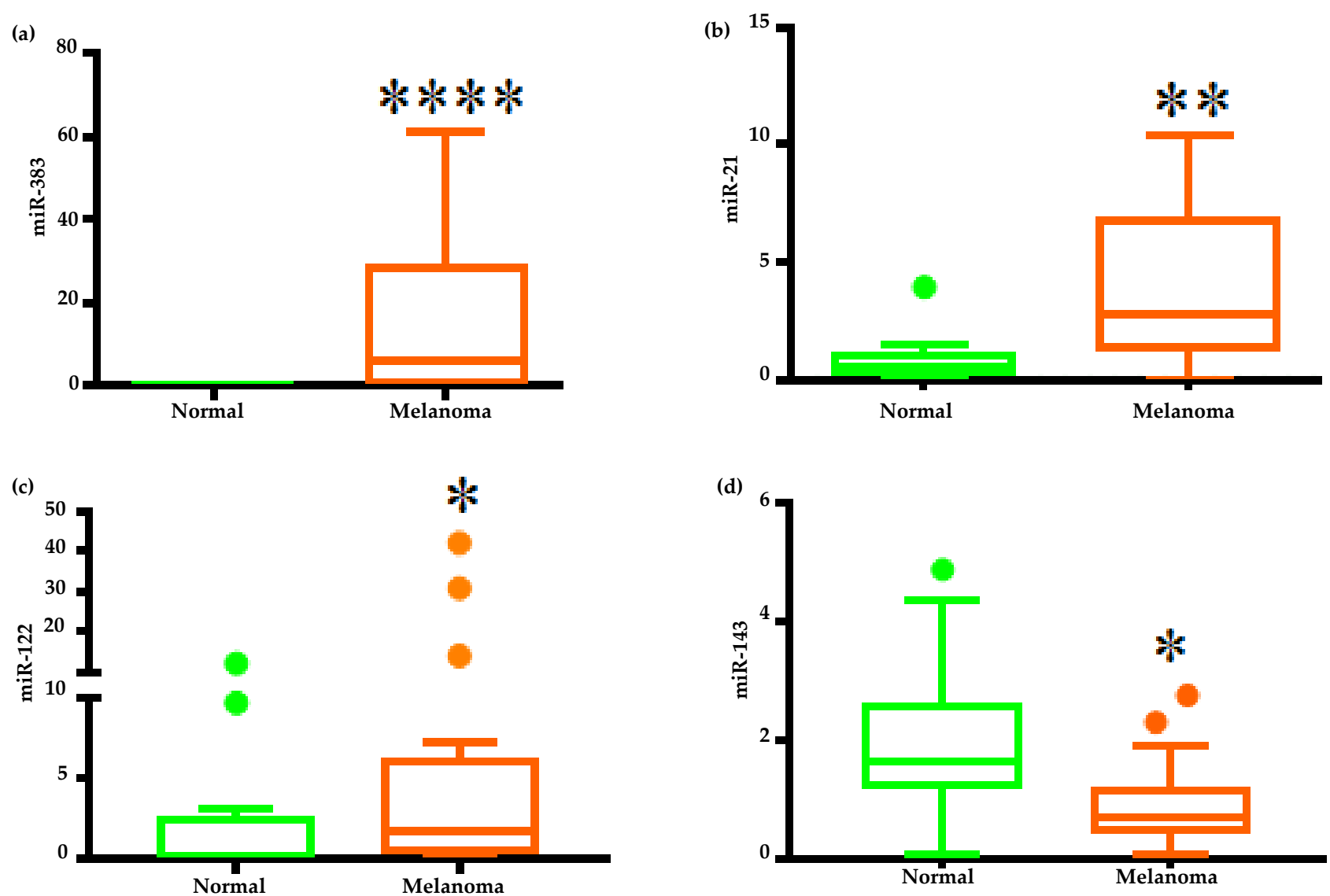
(c)



(d)



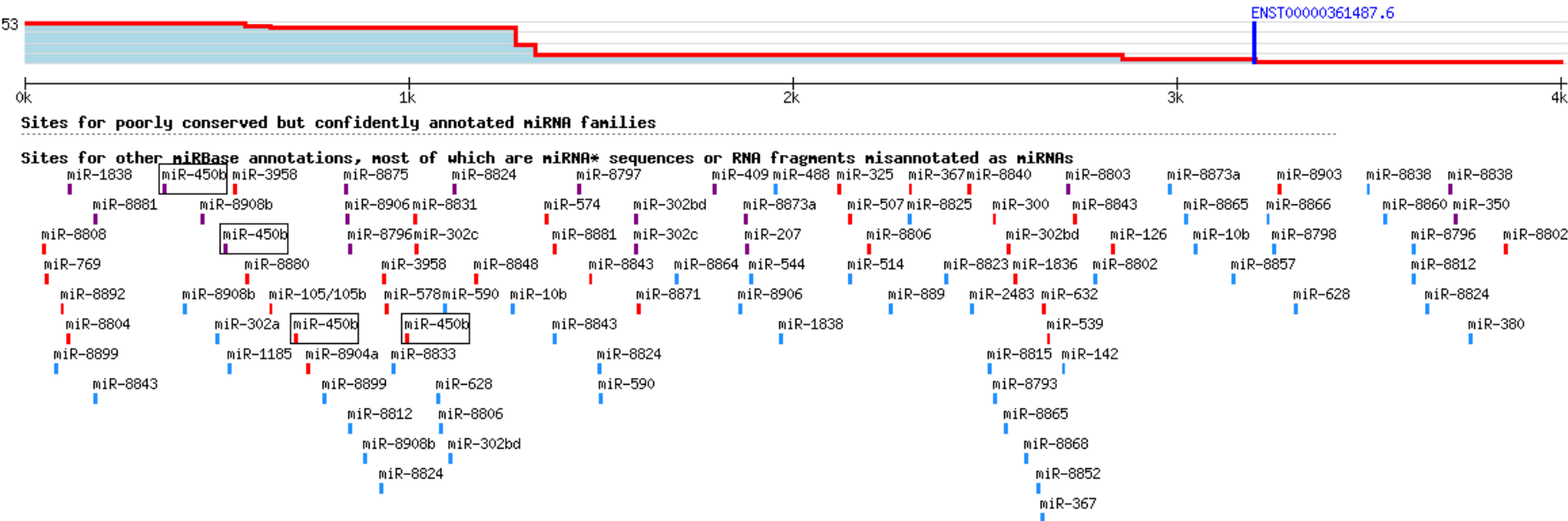
**Figure S1.** Profile of small RNA reads in canine oral melanoma: (a) Length distribution of clean reads in the normal and melanoma libraries; (b) Percentages of the clean reads annotated by the miRBase and Ensembl non-coding RNA databases; c) Unsupervised Euclidean hierarchical clustering by the miRNA normalized expression values in the normal and melanoma libraries, The colour scale (upper right) indicates the expression values; (d) Principal component analysis (PCA) of normal (green) and melanoma (pink) samples. The miRNAs read counts were normalized and transformed before PCA.



**Figure S2.** Differential miRNA expression in COM. (a-c) Relative expression of up-regulated miR-383, 21 and 122; (d) Relative expression of down-regulated miR-143. The Y-axis indicates the relative miRNA expression levels normalized against RNU6B (normal n=12, melanoma n=17; Mann-Whitney test followed by Tukey's test; \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.0001)

(a)

Dog PAX9 ENST00000361487.6 3' UTR length: 4013



	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	P <sub>CT</sub>
Position 380-387 of PAX9 3' UTR cfa-miR-450b	5' ...GCAAUUGUUGAGAUUUUGCAAAA... 3' UAAGUCCUUGUAUAACGUUUU	8mer	-0.22	96	-0.22	0.153	N/A
Position 544-551 of PAX9 3' UTR cfa-miR-450b	5' ...AGAUGACCUAUUUUGUUGCAAAA... 3' UAAGUCCUUGUAUAACGUUUU	8mer	-0.19	94	-0.19	0	N/A
Position 735-741 of PAX9 3' UTR cfa-miR-450b	5' ...UUUUUUUUUUUUUUUGCAAAG... 3' UAAGUCCUUGUAUAACGUUUU	7mer-m8	-0.03	50	-0.03	0.233	N/A
Position 1033-1039 of PAX9 3' UTR cfa-miR-450b	5' ...UUAAUGUGAAACGCUUUGCAAAG... 3' UAAGUCCUUGUAU-AACGUUUU	7mer-m8	-0.08	78	-0.07	0.153	N/A

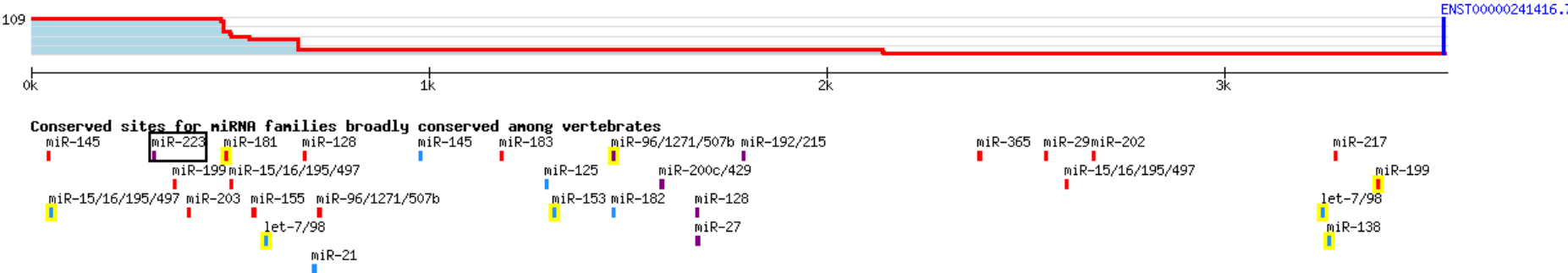
.....350.....360.....370.....380.....390.....400.....410.....420.....430.....440.....

Dog JCAACCUGAACUUUUGAAACGUGCAA---UUGUU---GAGA-UUUUGC-AAAAUC---AAUAAAGGAAACUACAU-----AUAGAAAA-----AAAA---AGUUUAUGCUAUACCCCUUAUAUCAAUAA-

Human JCAACCUGAACUUUUGAAAUUGUGCAA---UUGUU---GAGA-UUUUGC-AAAAUC---AAUAAAGGAAAUACUU-----AUAG-----AAAA---AAUUUAUGCUACACCCCUUAUAUCAAUAAU-

(b)

Dog ACVR2A ENST00000241416.7 3' UTR length: 3560



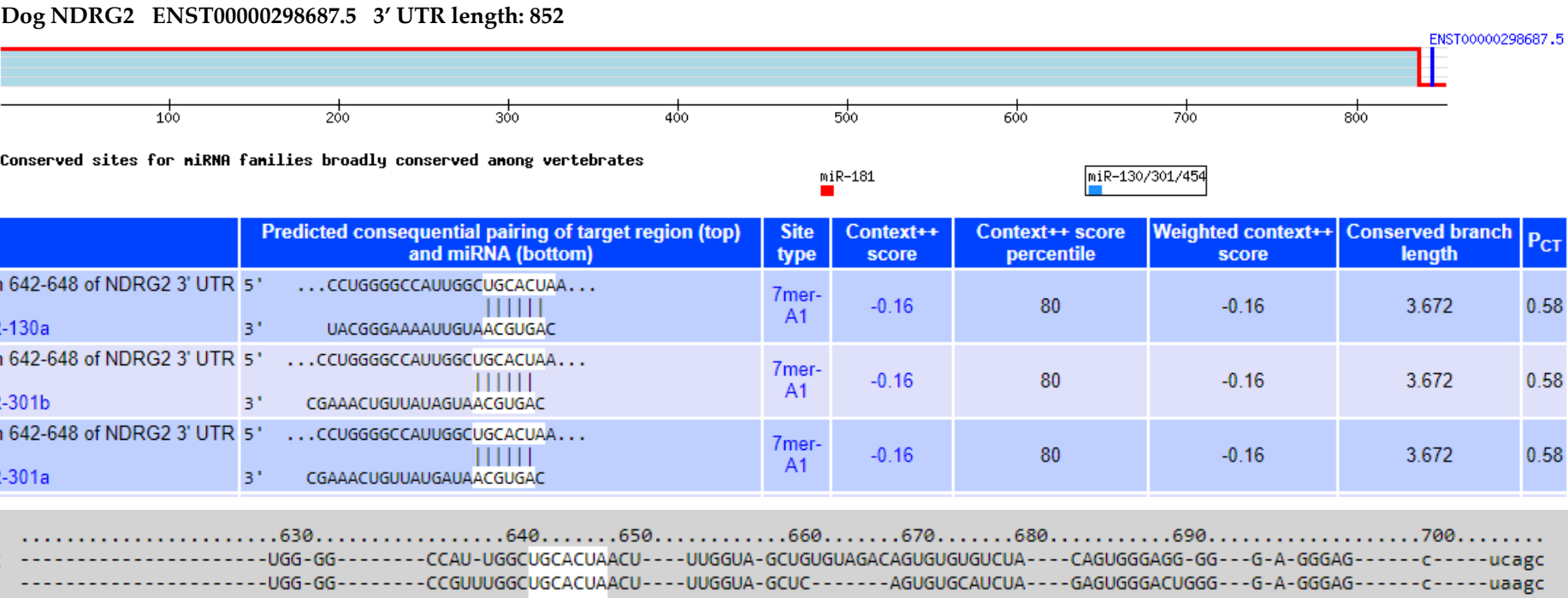
	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	P <sub>CT</sub>
Position 305-312 of ACVR2A 3' UTR	5' ...AAUCAAGUGUUUGAA-AACUGACA...	8mer	-0.46	98	-0.46	3.283	0.29
cfa-miR-223	3' CCCCAUAAACUGUUUGACUGU						

260.....270.....280.....290.....300.....310.....320.....330.....340.....350.....360.....

Dog ICUCCAAAU--C--AAGGAUC-UUUUGGACCU--GGCU-AAUCAAGUGU-UUG--AAAACUGACAUCAGAUUUC-----UAAUGUCUGUCGG--AA--GACACUAAUUC-UUAAAUGAACUACUGCUA----

Human ICUCCAAAU--C--AAGGAUC-UUUUGGACCU--GGCU-AAUGGAGUGU-UUG--AAAACUGACAUCAGAUUUC-----UAAUGUCUGUCAG--AA--GACACUAAUUC-UUAAAUGAACUACUGCU----

(c)



**Figure S3.** Predicted conserved target binding site of miR-450b-PAX9, miR-223-ACVR2A and miR-301a-NDRG2 from TargetScan: (a-c). Predicted binding sites of respective miRNA-mRNA are conserved between human and dog.